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AN ANALYSIS OF THE PHYTOTOXIC REACTION OF LODGEPOLE PINE FOLLOWING TREATMENT WITH FUEL OIL FORMULATED INSECTICIDES

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By

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INTRODUCTION

The use of fuel oil formulated insecticides by the U.S. Forest Service to treat tree boles of lodgepole pine, Pinus contorta Dougl., has been a common practice since the dawn of control programs. One such preventive spray program was initiated June 15, 1975, in Buffalo Campground, Targhee National Forest, Idaho, in an effort to protect living trees from attack of the mountain pine beetle, Dendroctonus ponderosae Hopk. (Klein, 1975). A total of 600 trees were treated throughout the campground with the insecticides Dursban, Lindane, and Sevin (200 trees per insecticide) at a 2% concentration in #2 fuel oil. Tree boles were completely sprayed from the ground up to the 25' level.

Unlike many other ground treatment projects, treated trees were permanently tagged and numbered for future reference in determining spray effectiveness. During the first re-evaluation study in late September 1975, a phytotoxic reaction in the form of a heavy "resin like" exudation from approximately the 25' level was noted on treated trees in all insecticide groups. Due to the low phytotoxicity ratings assigned to the respective insecticides (Dow Chemical, 1967; Demozay and Marechal, 1972; Union Carbide, 1967, the phytotoxic reaction appeared to be related to the carrier medium, #2 fuel oil.

Based upon these observations, studies were begun to determine the extent, seriousness, and nature of the phytotoxic reaction. The following report discusses the findings and observations of those studies.

METHODS

The approach used to study the cause and effect of the observed phytotoxic reaction was composed of three combined studies including field, microscopic, and chemical analysis.

In late June, 1976, a survey of all 600 treated trees was conducted to analyze and establish the reaction of individual trees to spray materials. Small bark sections were removed randomly around the tree bole near the 4.5' level with a hand ax. Trees were grouped into three classes on the basis of bark and crown vigor with care being taken to assure thorough sampling to facilitate accurate classification.

Class 1 was composed of those trees with dry phloem surrounding the entire tree bole and noticeable discoloration of the tree crown. Class 2 consisted of those trees exhibiting a vertical "stripping" effect between living and dry phloem tissue. The tree crowns in this class were healthy with little to no discoloration being present. Class 3 trees were those trees with living phloem surrounding the entire bole and a healthy crown. Trees were re-analyzed during the early part of August to confirm or reconsider prior class assignments. Long-term analysis was not possible due to a hazardous tree removal project being conducted in the campground which included the removal of treatment trees.

Another phase of field analysis consisted of the control spraying of living bark in specific bole areas (Fig. 1)[1] with water formulated mixtures of Dursban, Lindane, and Sevin (at relatively high concentrations of 25%,v/v), and #2 fuel oil. Experimental and nontreated control trees were randomly selected in an area adjacent to the 1975 project boundary. Tests were replicated twice with insecticide mixtures and three times with fuel oil with controlled amounts of material being applied in each case. Treated bark sections and 2 cm of surrounding, nontreated bark (including recent secondary xylem), were removed in late August and placed in a fixative composed of formaldehyde-acetic acid-50% ethanol, v/v.

Bark samples were prepared for thin sectioning and microscopic evaluation through dehydration in a seven step ethanol-tert-butyl alcohol-water series[2] and later infiltrated with paraplast (Saas, 1958). Sectioning of prepared tissues was accomplished using a rotary microtome with safranin O and fast green being employed as staining agents. Black and white 35mm photomicography was also employed to aid in tissue analysis.

A chemical analysis of exudate material from treatment trees was also conducted. Liquid exudate was collected from Class 1 trees near the 25' level by making a slash in the bark. Samples from adjacent nontreated trees were collected in a similar manner (although not necessarily at the 25' level) to serve as controls. The resulting flow of material from the wounds (significantly higher from treated trees) was collected in glass vials which were then sealed in plastic bags. Hardened exudate that was found on the bole of treated trees was also collected for analysis.

The brief analysis conducted consisted primarily of determining relative quantitative differences in the amount of resin and related compounds present compared to the amount of carbohydrates in the samples. Infrared spectroscopy, nuclear magnetic resonance, colorimetric (Pavia, et al., 1976), and double beam spectroscopic methods were employed during material analysis. Since no sample purification was attempted it should be noted that IR and NMR data served as quantitative indicators of the molecular species present in the various samples.

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[1]Figures and tables may be seen in the appendix.

[2]One step substituted absolute ethanol for dilute ethanol.

OBSERVATIONS

The phytotoxic reaction of lodgepole pine to fuel oil may be recognized to take two primary forms. The first is exudation of resinous materials on the bole of treated trees. Exudate material may be observed to originate from the point separating treated and nontreated bark (25' level in this case) leaving columns of hardened exudate as the material flows progressively down the bole of the tree (Fig. 2). This type of exudation was the first indicator of a phytotoxic reaction. Another form of exudation allows easy visual identification of affected trees due to the yellow cast it gives to the tree bole (Figs. 3 & 4). Upon closer observation the yellow cast is found to be a result of the formation of resinous droplets that were exuded over the treated bole surface and hardened prior to any vertical movement (Fig. 5). This form of exudation may encompass the entire tree bole where spraying has been conducted but may vary in the relative amounts that are present.

The second phytotoxic reaction is observed as necrosis of bark, cambial, and secondary xylem tissue (Fig. 6). The associated discoloration (yellow-brown to black) often extended extensively into the secondary xylem ($\triangleq$ 2/3 bole radius) and was always observed to affect inner and outer bark tissues. Trees exhibiting this condition were often found to have a saprophytic, powdery mildew-like (no positive identification was attempted), fungus growing in "pockets" formed where necrotic bark had separated from the cambium.

The effect of spray materials on treated lodgepole pine in Buffalo Campground may be seen in Table 1. The results presented would seem to indicate tree mortality to be related to the insecticides applied. Through microscopic studies of experimentally treated bark, however, this has been found not to be the case. Bark samples treated with high concentrations of water formulated insecticides (to be discussed later) showed no significant anatomical deviations from the anatomy of non-treated bark. Fuel oil treated bark samples showed definite tissue deterioration.

A possible explanation for the results in Table 1 may lie in a relationship between tissue mortality (Classes 1 and 2) and the maximum air temperature at the time of spray application. A breakdown of the data in Table 1 showing the mortality of treated tissue for the first 15 days of spraying in relation to temperature and wind may be seen in Table 2. Graphing these data, a relationship between mortality and the maximum air temperature (Fig. 7) appears plausible. Removing those points when the maximum wind speed was equal to or greater than 20 mph, this relationship becomes much clearer (Fig. 8) with the regression correlation going from $r=0.57$ to $r=0.98$, respectively. The regression correlation value would indicate the wind to be an important variable in this relationship. The importance of this variable is further

substantiated when considering the type of spray applicator being employed. Due to the extreme atomizing affect the type of nozzle being employed had on the spray material, high wind often required deviations from the normal mode of application to reduce the amount of drift. Such techniques undoubtedly affected the amount of spray material being applied to the tree bole when compared to standard application techniques when wind was not a factor. Unfortunately, since wind and temperature data were not available throughout the day, we are forced to group trees into catagories of environmental absolutes.

Nevertheless, since a relationship does seem to exist, the least squares method of finding the line of best fit may be applied to those points in Fig. 8. The resulting line may be mathematically represented by:

$$Y = 1.88x - 55.41$$

where Y equals the percent mortality and X equals the maximum temperature in degrees fahrenheit.

This formula presents a most interesting application. Should fuel oil be employed in a future project, a theoretical value of expected mortality could be projected by inserting the expected daily maximum temperature for X and then solving for Y. However, since a strict testing of this formula's value has not been conducted in relation to the stand's composition profile (Bergen, 1974), wind and temperature variables were considered only in daily absolutes, and the idea of planning for mortality in a protective spray application project is not probable, the actual usefulness of this formula may be somewhat academic.

The stripping effect between living and dead tissues on treatment trees is also of interest. An extensive analysis of only Class 2 trees was conducted in early August, 1976, with special attention being given to the respective aspects of living and dead tissue. Studies showed conclusively that no relationship exists between the north, south, east, or west aspect and the condition of tissues. Thus, the observed stripping affect occurs randomly around the tree bole. A less exhaustive study of necrotic tissue in relation to bark thickness also proved negative. Circumstantial evidence relating the stripping effect to wet tree boles at the time of spray application does exist but fails to explain the overall situation adequately. The most probable cause seems to relate stripping to variations in spray application (e.g., missed or under-sprayed areas and overlapping during application), and, to a lesser extent, a combination of other factors including tree vigor, resistance of bark to fuel oil penetration, and the amount of water on the bole at time of spraying.

Results of experiments designed to determine the phytotoxicity to lodgepole pine from the individual insecticides as well as fuel oil demonstrated that the cause of the observed phytotoxic reaction was directly related to fuel oil. Microscopic evaluation of the tissues treated with water formulated insecticides found no cellular abnormalities with a functional cambium being present.

Fuel oil treated bark samples exhibited distinct tissue abnormalities with cellular degeneration being common. Strand (axial) parenchyma exhibited definite signs of necrosis with cell wall breakdown being representative. In advance cases of cell degeneration, cells appeared to acquire the appearance of a solitary strand with individual cells not being distinguishable. Ray parenchyma was seen to have similar cellular degeneration to strand parenchyma. Sieve cells that were affected lacked sieve areas and distinct cell wall boundaries were not recognizable. The cambium did not show distortions of the nature previously described but was nonfunctional. Comparisons made between treated and adjacent nontreated tissue, showed a substantial reduction of additive cell replication by the cambium in the treated areas. The loss of fusiform initials without effective replacement appeared to be the cause for the noted reduction in cell addition. Secondary xylem was penetrated by fuel oil via ray parenchyma. No cellular disorders were observed in this tissue at the time of study, but study of tissues collected from the 1975 project indicate eventual breakdown of tracheid cell walls. The breakdown of tracheids occurs in a vertical direction from the ray parenchyma and is believed to occur in the latter or early part of the growing season. A more detailed study of the anatomy of treated tissues (Rogers, 1976) should be referred to for further descriptions.

The mechanism of cellular degeneration resulting from contact with fuel oil remains to be determined with brief studies indicating a breakdown of cell wall components other than those with celluloid composition. With conclusive chemical evidence lacking, this area of study should be considered in greater detail in future studies for a better understanding of the mode of fuel oil penetration and affect on treated tissues.

The chemical nature of exudate material collected at the 25' level was found to be high in resin content with no carbohydrates being detected. Exudate that did not eminate from the 25' level, but originated from the treated bole surface was found to contain both resins and carbohydrates. Carbohydrates were found to be present in a concentration of approximately 1×10^{-3} M per gram of exudate. The bulk of the exudate was composed of resinous compounds.

These data would indicate that the buildup of resin at the 25' level between living and dead tissues was a wound response by the tree (Esau, 1965), while exudate from the treated bole surface may have resulted from the combination of carbohydrates that had been in cells at

the time of their death and resins that were exuded by a wound response. The relatively large amounts of resin exudate from the 25' level may have been a reflection on the adjacent living tissue's resin production capabilities since resin exudation was noticeably reduced away from a living tissue area.

The stimulation of high resin production occurred only at vertical breaks between living and necrotic tissue at the 25' level in trees with fully necrotic bark tissues. Exudation between horizontal breaks of living and necrotic tissue in Class 2 trees was not observed while lesser exudation between vertical breaks in strip trees was only rarely observed. Exudate with a carbohydrate component was observed on necrotic bark of strip trees but in lesser amounts than on Class 1 trees.

SUMMARY

The results of this study have established that the use of fuel oil as a carrier medium for insecticides, when bark saturation techniques are employed using living trees, should be discontinued. While water formulated insecticides are not considered to penetrate the bark as thoroughly as those formulated with fuel oil (Davis, 1976), the resulting phytotoxic reaction and tree mortality makes its use impractical. Fuel oil phytotoxicity is also known to be greater when mixed with many insecticides (Davis, 1976).

A phytotoxic reaction by lodgepole pine may be recognized as resin exudate and/or necrotic outer bole tissues. Exudation may be strictly resinous substances or a mixture of resins and carbohydrates.

Fatal phytotoxicity in lodgepole pine to fuel oil may be related to the air temperature at the time of application. With the type of applicators being used in the 1975 spray project, wind was also found to be an important factor in this relationship.

The observed phytotoxicity was a direct result of cellular and tissue breakdown. Strand and ray parenchyma in the phloem were often distorted beyond ready identification. Sieve cells lacked sieve areas and the cambium in treated areas was nonfunctional. Secondary xylem was often necrotic and extensively penetrated by fuel oil. This penetration was probably facilitated by xylem ray parenchyma. Distortion of cells appeared to result from cell wall breakdown by an undetermined mechanism.

With the recognition of a phytotoxic reaction of orchard trees to bark saturation with fuel oil formulated insecticides (Davis, 1976) and now conclusive evidence of a similar reaction by lodgepole pine, it is clear

that fuel oil has seen its day for spray applications of the nature described. Hopefully, the 1975 spraying failure will serve as a concrete warning against the future use of fuel oil and similar substances in saturation spraying without carefully controlled pre-testing of their effects on the target species.

A P P E N D I X

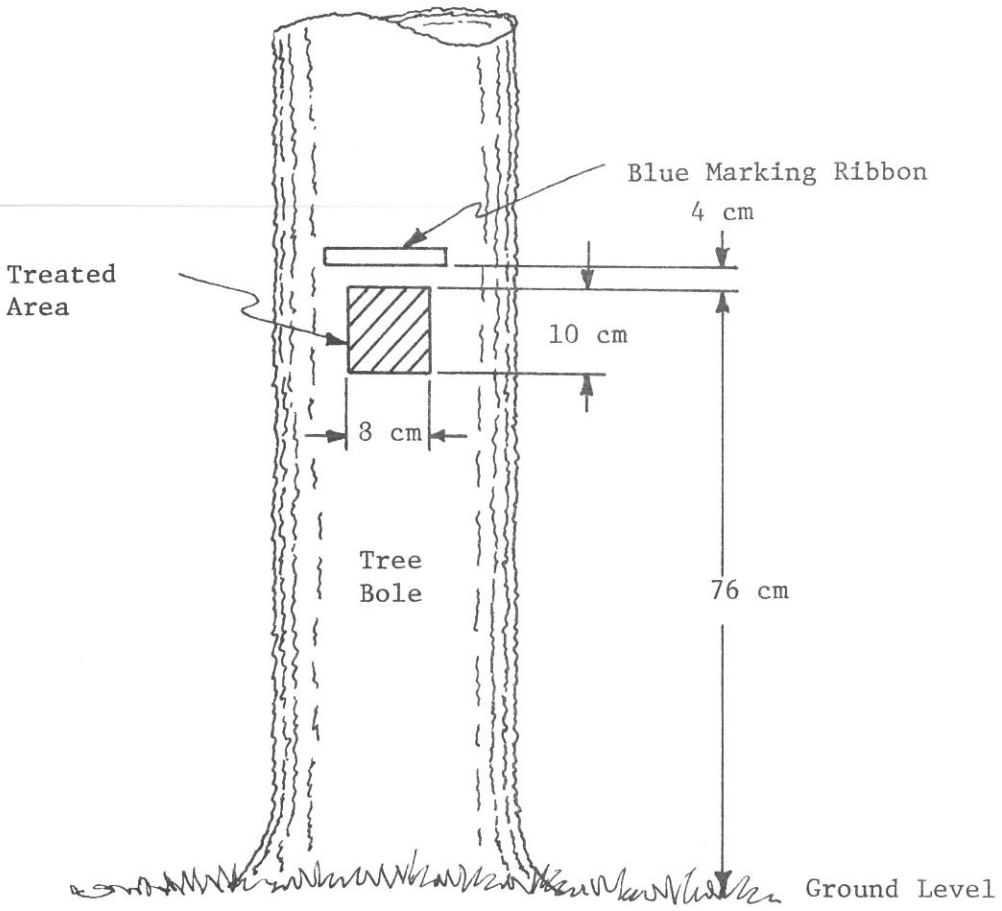

Figure 1. Illustration of standard technique applied to establish experimental bark treatment areas.

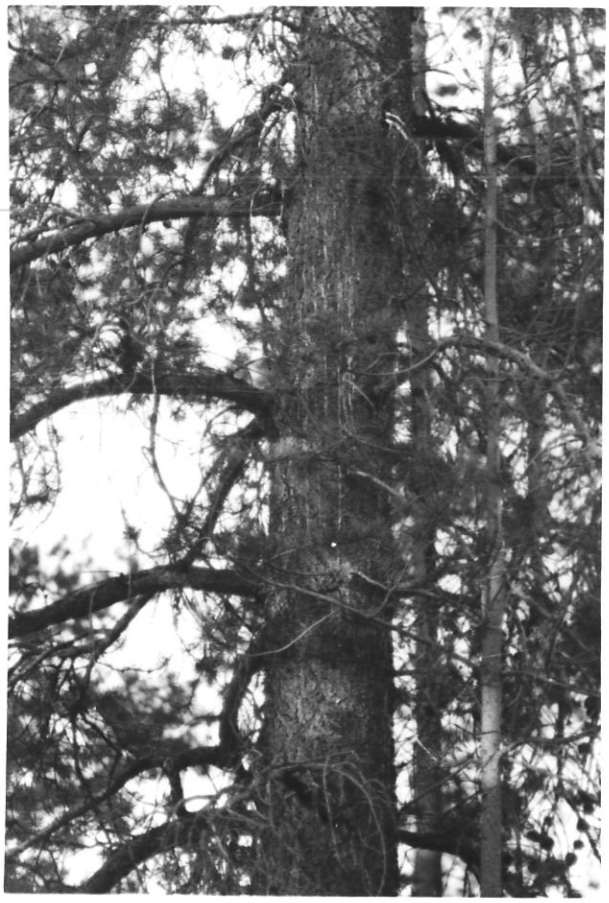

Figure 2. Exudation from the 25' level of a treated tree bole. Note vertical flow.

Figure 3.

Photograph of nontreated lodgepole pine bole.

Figure 4.

Photograph of treated lodgepole pine bole.

Figure 5. Closeup of Figure 4 showing beaded appearance of exudate and lack of vertical flow. Note metal tag marking treatment tree in the upper center of the photo (attached to tree bole).

Figure 6. Necrotic secondary xylem in the cambial region (necrotic phloem removed). Note degrees of discoloration.

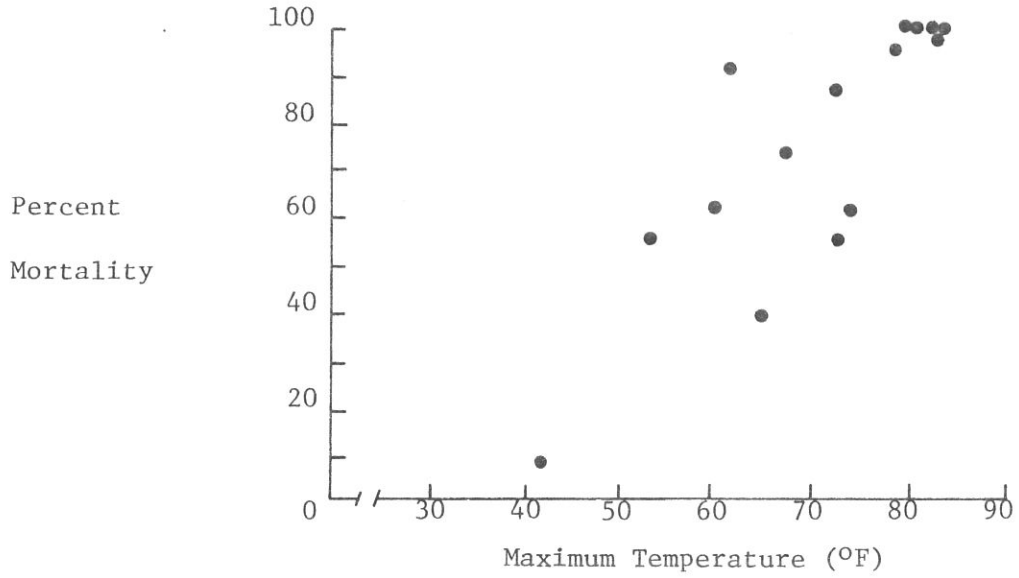

Figure 7. Graphic representation of data in Table 2.

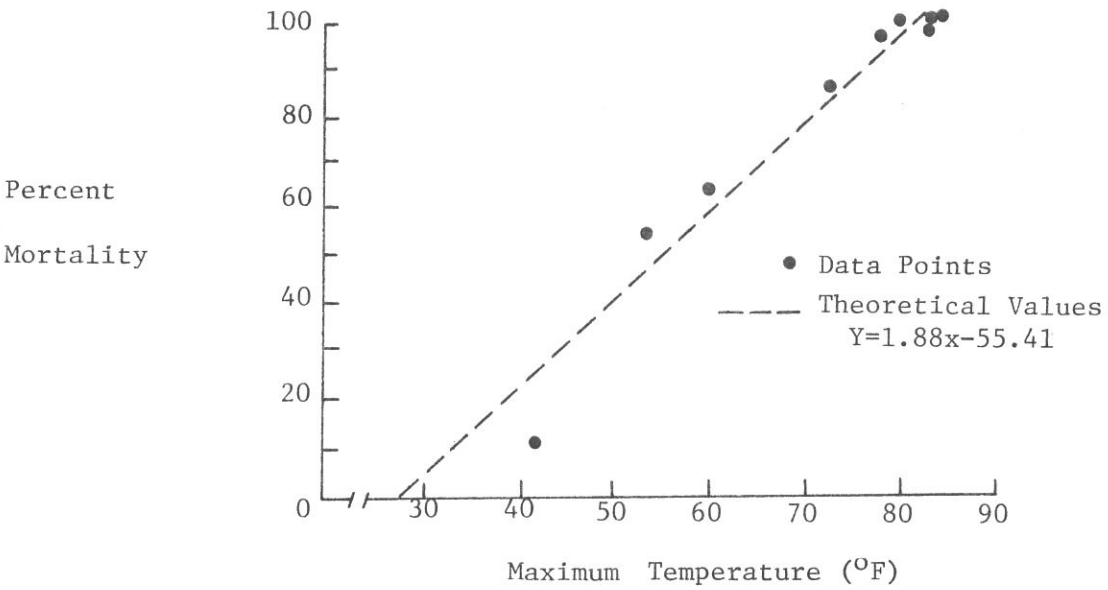

Figure 8. Graphic representation of data in Table 2 when wind is less than 20 mph. Theoretical value line also shown.

TABLE 1.

INSECTICIDE GROUP	TREE CONDITION[1]		
	DEAD (Class 1)	LIVE STRIP (Class 2)	LIVE (Class 3)
DURSBAN	84	15	1
LINDANE	73	19	8
SEVIN	56	10	34

[1]Given in percentage

TABLE 2. Breakdown of data in Table 1 showing temperature and wind conditions during first 15 days of spray application.

Date (1975)	Maximum* Temp. (°F)	Maximum* Wind (MPH)	Phloem Condition[1]	
			Mortality Present	Live
June 15[2]	73	25	55	45
June 16[2]	65	21	40	60
June 17[2]	53	17	57	43
June 24[2]	74	28	61	39
June 25[2]	42	9	10	90
June 26[2]	60	26	62	38
June 27[2]	62	31	91	9
June 28[3]	67	29	73	27
June 29[3]	73	13	86	14
June 30[4]	80	21	100	0
July 1[4]	79	15	95	5
July 2[4]	85	16	100	0
July 3[5]	84	14	98	2
July 4[5]	83	11	100	0
July 5[5]	84	6	100	0

*Climatological Data, National Oceanic and Atmospheric Administration, 78-79/no. 6&7, 1975.

[1]Given in percentage

[2]Only Sevin treated trees

[3]Sevin and Lindane treated trees

[4]Only Dursban treated trees

[5]Dursban and Lindane treated trees

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